SOME STUDIES OF THE ELECTROLYTE EQUILIBRIUM OF THE EHRLICH ASCITES TUMOR OF MICE

Guy Mead McKhann

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TABLE OF CONTENTS

INTRODUCTION

It has been almost forty-five years since Henderson adapted the mass action dissociation laws of Arrhenius and Ostwald to solutions of weak acids and their salts. Henderson's equation, adapted to its logarithmic form by Hasselbach, is shown as it applies to the carbonic acid-bicarbonate systems in the body (1).

$$pH=pK' + \log \frac{[HCO_2]}{[H_2CO_3]}$$

Since that time, there have been great advances in the understanding of the regulation in the factors of this equation by the body. The respiratory regulation of carbonic acid has been intensively investigated by many authors and recently reviewed by Brown (2) and Gray (3). The direct renal regulation of bicarbonate, and the indirect effects on bicarbonate of the renal regulations of sodium, potassium, chloride, hydrogen and other ions have been intensively investigated with many interesting, if occasionally conflicting, results.

There remain, however, a number of poorly understood mechanisms, both proposed and real, which

numerous authors have labelled as "the extra-renal factors". These "extra-renal factors" refer to the possible exchanges of electrolytes between the extracellular compartment and two large reservoirs of electrolytes, the bone and the intracellular compartment. Some of the possible exchanges are depicted in Figure I.

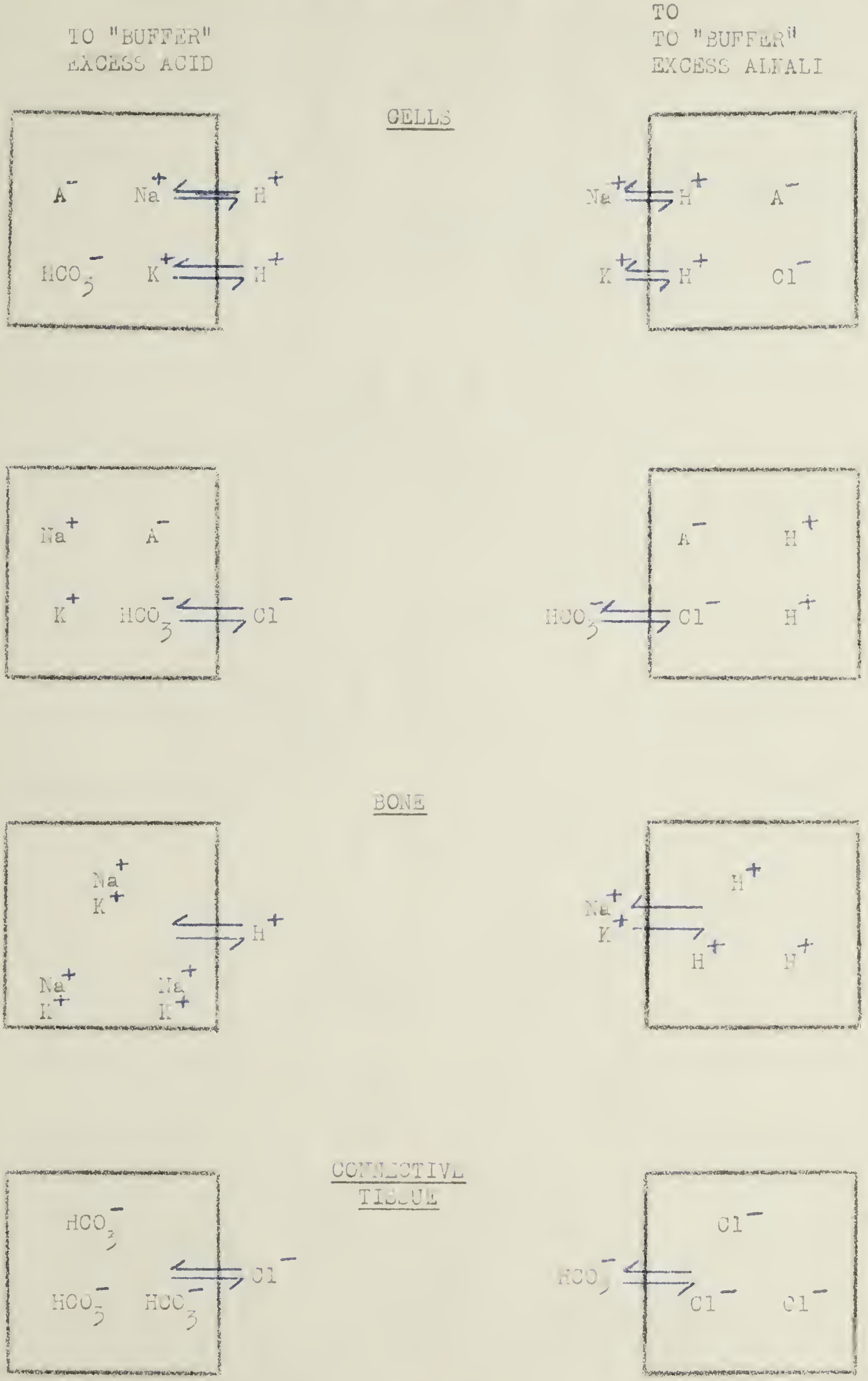
The proposed exchanges of cations have been demonstrated by several investigators. Cooke (4, 5) and his associates have demonstrated exchanges between intracellular potassium and extracellular sodium and hydrogen in the production and correction of alkylosis. Pitts (6) and Giebisch (7) have noticed similar exchanges, involving both intracellular sodium and intracellular potassium, in respiratory acidosis and alkylosis. Further, a possible mechanism for the exchange of bone cation with the cation of the extracellular fluid has been proposed and demonstrated by Bergstrom and Wallace (8, 9).

On the other hand, the possible anion exchanges have not been adequately evaluated. Although several authors have suggested that some exchange might occur between the bicarbonate and chloride of tissue and extracellular fluid (10), no such exchange has ever been adequately demonstrated either in vivo or in vitro with any tissue except the erythrocyte.

Until recently, the chloride ion was considered

FIGURE I

POSSIBLE EXCHANGES OF IONS



Adapted from Pitts, R., F., Tr. Conf. on Renal Function, Josiah Macy Jr. Foundation, New York, 1952, p 12.

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to be in diffusion with the plasma, and solely extracellular in distribution. In fact, it was on this assumption that Darrow and his associates used chloride ion to measure the extracellular space (11). However, it is now known that chloride ion is present in small quantities in some tissues, (10) and that there is a third depot of chloride described by Cooke (12) as the "extra-extra cellular space", which is considered by many to be the connective tissue and skin. However, in the mammal, there is one tissue that has a high intracellular chloride content, the erythrocyte. Not only is the erythrocyte permeable to chloride, but also reciprocal exchanges between the erythrocyte anions and the anions of the extracellular fluid can and do occur. Exchanges between cellular bicarbonate and extracellular chloride, the well known "chloride shift", were first described by Hamburger, Fridericia (13), and Van Slyke (14) about 1915. Although this exchange is familiar, a short discussion is necessary at this time because this exchange forms the basis of the present study.

When considering the "chloride shift" it is helpful to remember that when one is dealing with two solutions separated by a semi-permeable membrane three basic laws of physical chemistry must be met, before equilibrium can be established (15, 16).

1) The positive and negative charges on each side of the membrane must be in balance.

2) According to the Donnan Equilibrium, the distribution

of the diffusible ions is a constant proportion when the system is at equilibrium. For blood, this distribution may be expressed as:

$$\frac{(Cl)c}{(Cl)p} = \frac{(HCO_3)c}{(HCO_3)p}$$

3) Total osmolar concentrations on each side of the membrane must be equal.

The application of these laws to the "chloride shift" can be demonstrated in a much more understandable fashion by a diagram than by any attempt at a detailed description. However, certain features of this exchange should be stressed.

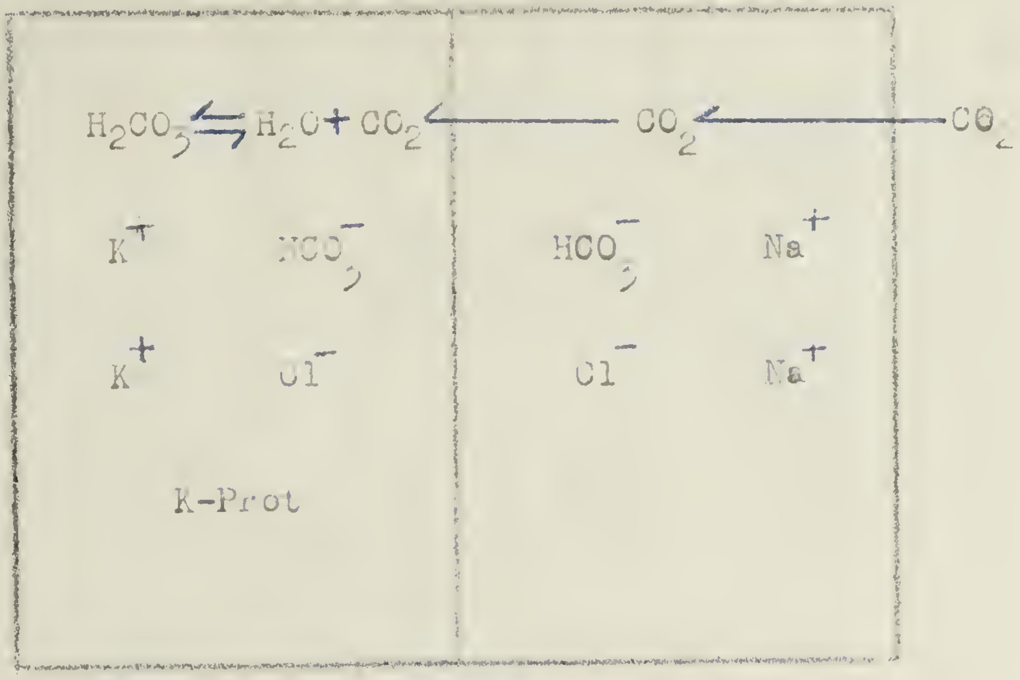
a) The overall effect of this exchange is to allow the hydrogen ions, resulting from the dissociation of carbonic acid, to be buffered by the strong intracellular protein buffers rather than by the very weak plasma buffers.

b) The increase in intracellular bicarbonate upsets the limits imposed by the Donnan Equilibrium. Consequently, there must be either a shift of bicarbonate to the extracellular fluid, of chloride to the intracellular fluid, or both.

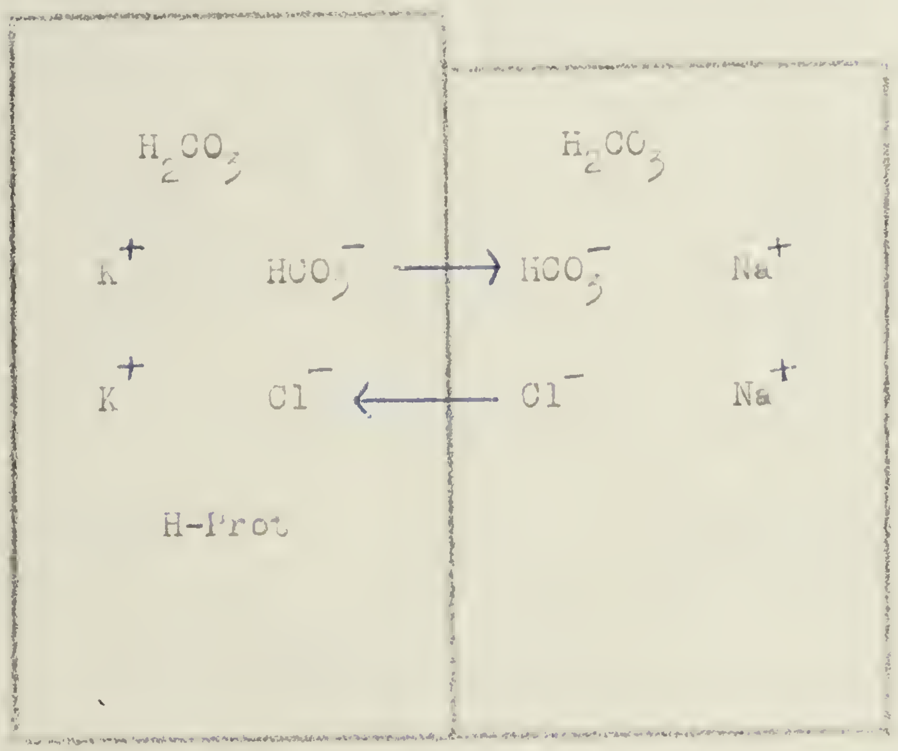
c) If the law regarding electrical neutrality is to be met, bicarbonate ion can shift intracellularly only if it exchanges for another available (chloride) anion, or if it

FIGURE II

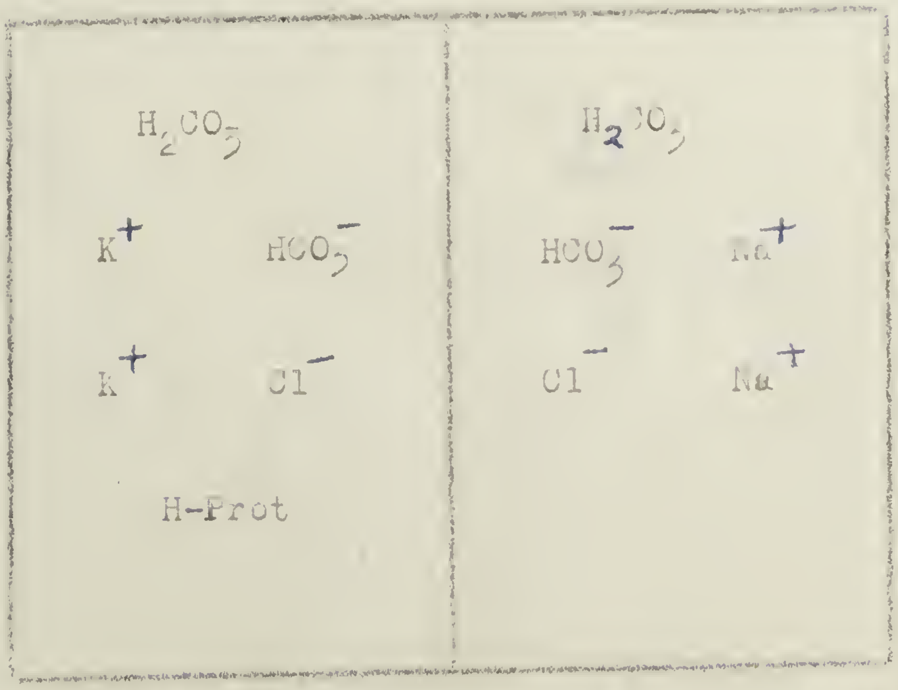
THE "CHLORIDE SHIFT"



Original state of equilibrium



Shift of HCO_3 to preserve Donnan Equilibrium



Shift of H_2O to preserve osmotic equilibrium

is accompanied by a cation. Since the erythrocyte is impermeable to the chief intracellular cation, potassium, it is more probable that an exchange for extracellular chloride occurs.

d) Although some intracellular bicarbonate exchanges for extracellular chloride, the net effect is an increase in both intracellular bicarbonate and intracellular chloride. This net increase results in an increase in total intracellular osmolarity.

e) Finally, there is a shift of water to the intracellular compartment to re-establish osmotic equilibrium.

In this series of experiments, the cells of the Ehrlich ascites tumor of mice are exposed to high tensions of carbon dioxide in an attempt to demonstrate the "chloride shift" for a biological system other than the blood. The adaptability of the cells of the Ehrlich ascites tumor to the investigation of problems of electrolyte equilibrium has not been fully explored. However, it is conceivable that high tensions of CO_2 might affect other functions of an actively metabolizing cell besides the maintenance of electrolyte equilibrium. On the other hand, it is possible that any anion "transfer system" that might exist in the erythrocyte might better be demonstrated in a system of more active cells.

The Ehrlich ascitic tumor of mice is a biologic phenomenon which is sufficiently unique to warrant a

discussion at this time. In 1932, Lowenthal and Jahn (17) observed that the inoculation of cells of the Ehrlich carcinoma of mice into the peritoneum of a mouse was not always followed by a solid tumor of the peritoneum, but occasionally by an hugh ascites. This ascites appeared after a three or four day "latent" period, and consisted of twenty to thirty cubic centimeters of a milky or bloody fluid which had a high content of cells. The passage of this newly-formed "ascitic" tumor could easily be performed by the intraperitoneal inoculation of a small amount of the ascites fluid into another mouse. If the proper dilution and size of inoculation were used, 100% "takes" could readily be obtained.

Dietrich and Schutzinger (18), and Lettre (19, 20) have studied the cells of the Ehrlich ascitic tumor and found that these cells "float" freely in the peritoneal cavity, growing and dividing independently at a very rapid rate. Lettre (20) has calculated that each cell divides about once a day, with the actual mitosis requiring about fifteen minutes. Dietrich and Schutzinger (18) consider these cells to be maximally differentiated and to possess maximal proliferative energy.

The stability of these cells is remarkable. Unlike the cells of solid tumors, the ascitic tumor cells do not show signs of degeneration in vivo. Hoagen and Kruckberg (21), and Luecke (22) have made observations on

the in vitro stability of these cells at various temperatures. Both groups of observers found that these cells were still intact after five hours at room temperature.

The possible experimental uses for these living, actively metabolising cells are manifold. Lettre (20), Klein (23), and others have used this ascitic tumor to evaluate the anti-mitotic activity of various compounds. When the inoculation contains a standard number of tumor cells, the increase in weight of the animal, the amount of ascites fluid produced, the duration of the survival of the animal, and the cytology of the tumor cells can all be accurately predicted. Using these criteria, an inhibitory effect on the growth of this tumor has been demonstrated for colchicine, cortisone, nitrogen mustards, 6-mercaptopurine, and other compounds (23, 24).

Klein and his co-workers (23) have studied the nucleic acid content of these cells and found that the pentosenucleic acid content was constantly five times the value reported for non-tumorous exudate cells and that the desoxypentosenucleic acid content was about twice the normal value of mouse cells. These authors correlate the rapid growth rate, the high uniform nucleic acid content, and the reproducibility of their findings with the good nutritional status of the in vivo environment of these cells. Christensen and Riggs (25) found that freshly drawn ascitic cells

contained amino acids in concentration less than or equal to that of the suspending fluid; but, on standing, these cells concentrated amino acids with great activity. Added single amino acids were concentrated with an activity not observed previously for mammalian cells. These authors feel that this highly concentrative uptake might help to explain the competitive success of the neoplasm.

Lucké and Parpart (22) have recently studied the permeability of the ascitic tumor cells and compared the tumor cells with the mouse erythrocyte. These authors feel that these ascitic tumor cells are particularly adapted for quantitative determinations of osmotic phenomena and relative permeability because these cells can be obtained in large number as uninjured cells of a single type, are free to undergo volume changes which can be accurately measured, and because these cells can be kept outside the body for hours without undergoing degenerative changes. There are very few other types of animal cells which share these unique properties; the erythrocyte and the eggs of certain marine invertebrates being the most notable.

This unique biological system offers excellent opportunities for the investigation of not only amino acid metabolism, but also electrolyte equilibrium. It is conceivable that this biological system of "active" cells might be better suited than a system of "passive"

erythrocytes for the investigation of the electrolyte equilibrium between living cells and the surrounding extracellular fluid.

MATERIALS AND METHODS

Both male and female Swiss mice, weighing between twenty and thirty grams were injected intraperitoneally with .1 to .3 cc. of fresh tumor fluid. The tumor fluid contained between five and fifty million cells per cc. The mice regularly developed large amounts (five to twenty cc.) of ascitic fluid in from seven to ten days, and usually died in two or three weeks.

This method of implantation resulted in ninety percent "takes", with 25% of the animals developing subcutaneous tumors at the site of implantation. No differential counts were done to determine what percentage of the cells were "normal" cells such as leucocytes, histiocytes, etc. However, several authors have stated that about ten percent of the cells are not tumor cells (24). Hemorrhagic fluid was observed as the animals matured, being constantly present after the sixteenth day of tumor-bearing. The use of some hemorrhagic fluid for experimentation was unavoidable, but grossly hemorrhagic fluid was excluded when evaluation of the chloride shift was being performed.

<u>Handling of the ascitic fluid</u>: The mice were anesthetized lightly with ether, and the ascitic fluid was removed anaerobically from the peritoneal cavity of the mouse by a perforated polyethylene tube inserted percutaneously through a number fifteen needle. Between five and fifteen cc. of

fluid could usually be obtained. The collected fluid, with a small amount of Heparin added, was placed under oil prior to analysis. Pooled samples of fluid were often used for experimentation. Experimentation and analyses were usually carried out within an half hour after removal from the host.

Hematocrits: Hematocrits were determined, usually in duplicate, by the Wintrobe method. No measurement was made of the amount of "trapped" supernatant fluid, but it is doubtful if it exceeds the 2% calculated for blood.

pH: The pH of the whole ascitic fluid, and occasionally of the supernatant fluid, was determined with a Beckman pH meter, equipped with an anaerobic calomel glass electrode. With this method, only $\frac{1}{2}$ cc. of fluid was required for determination. The standard error was .02 units. The pH determinations were carried out in a room maintained at 38° centigrade in order to avoid the use of factors for temperature correction.

Electrolyte composition: The electrolyte composition of the supernatant fluid, and packed cells was evaluated by methods previously reported for this laboratory, except for the intracellular carbon dioxide content. Intracellular carbon dioxide was calculated by the formula:

$$[CO_2]_t = [CO_2]_s \times V_s + [CO_2]_c \times V_c$$

$[CO_2]_t$ and $[CO_2]_s$ refer respectively to the CO_2 content of the

whole ascitic fluid, and the CO_2 content of the supernatant fluid after centrifugation. V_s and V_c refer to the respective volumes of supernatant fluid and cells as determined by hematocrit. Peters and Van Slyke (1) suggest that the accuracy of this method is within 2%. This formula can also be used for the calculation of intracellular bicarbonate if the values for the bicarbonate of the whole fluid and of the supernatant are used. Tables I and II indicate the extracellular and intracellular composition of the ascitic fluid.

Gas analysis: The tensions of carbon dioxide and of oxygen were measured by the Scholander technique.

METHODS

EFFECT OF HIGH TENSIONS OF CO_2

The methods used for evaluation of the effect of different tensions of CO_2 on the tumor cells were similar to those used for the classic experiments on blood (13, 14).

Preparation of material: About 15 cc. of ascitic fluid were withdrawn anaerobically from the peritoneal cavity into an heparinized syringe. Equal aliquots were then placed in "double-chambered" tonometers and equilibrated with gas mixtures containing oxygen, nitrogen, and carbon dioxide. The actual tensions of various gases were not measured until the equilibrations were completed.

Equilibration: The technique employed was that described by Austen et. al. (26) as their "First Saturation Method" with a double-chambered tonometer. The double-chambered tonometer consisted of two single stopcock tonometers; a large one with a capacity of 300 cc., and a small one with a capacity of 30 cc., connected by a short length of rubber tubing. After equilibration, the liquid phase could be separated from the gas phase by allowing the liquid to run into the small chamber, and then cutting the connecting tube between two clamps.

The actual equilibration was accomplished by placing the double-chambered tonometers in a constant temperature water bath, maintained at 37.0° C. ± .5° C. The tonometers were clipped onto "spokes", which radiated out from an axle placed in the center of the tank, and rotated at about 20 RPM for thirty minutes. An increase of the gas pressures within the tonometer above atmospheric pressure was minimized by frequently opening the stopcock of the small chamber near the surface of the water.

While the tonometer was still under water, the liquid was drained into the small chamber, and the connecting tube was clamped and "cut". The liquid in the small chamber was kept under positive pressure by attaching a mercury levelling bulb, to the tubing and slowly forcing the remaining gas out through the stopcock. The small chamber was then removed from the water and samples were drawn anaerobically for the determinations indicated in Table III.

The gas remaining in the small chamber was forced out in a similar manner for analysis on the Scholander apparatus.

DETERMINATION OF THE SOLUBILITY OF CARBON DIOXIDE

The symbol α refers to the solubility coefficient of CO_2 in a liquid, and indicates the number of cubic centimeters of a gas, reduced to standard conditions of 0° and 760 mm., which are dissolved in 1 cc. of a solution in equilibrium with pure CO_2 at a pressure of 760 mm. The technique for determination of α was that described by Van Slyke, et. al. in 1924 (27).

Preparation of material: Ascitic fluid was withdrawn from several mice and pooled. The pooled fluid was then acidified to a pH of 3.5 by the addition of a small amount of IN HCl.

Saturation: The acidified fluid was equilibrated with 100% CO_2 in the manner described previously.

Analysis: The CO_2 content was determined in duplicate in the manometric gas apparatus of Van Slyke and Neill. A special 2 cc. pipette was employed which allowed transfer of solution from the small chamber to the Van Slyke apparatus without exposing the fluid to oil or air. This pipette is similar to the one described by Van Slyke and Neill (28).

Gas tensions: The tension of CO_2 used for equilibration was determined by the Scholander method.

CALCULATIONS AND RESULTS

GENERAL CONSIDERATIONS

It is well known that erythrocytes can survive for many hours outside the body. The stability of the cells of the Ehrlich ascites tumor reported in the literature, has already been mentioned. Lucké and Parpart (22) have shown that the permeability of the Ehrlich tumor cells to hypertonic sodium chloride does not change despite a five hour lapse between removal from the host, and experimentation. Those authors state that the persistence of the same state of all permeability proves the absence of cell injury.

Further, in this series of experiments, the pH of the tumor fluid was quite constant. Frequent determinations of pH on the same sample over a forty-five minute period failed to reveal any significant change in pH. The pH determinations on whole ascitic fluid and on the supernatant fluid of aliquots which had been either centrifuged or allowed to sediment were the same. These constant pH findings suggest that these cells remain intact while undergoing the procedures preparatory to analysis.

COMPOSITION OF SUPERNATANT FLUID

Table I indicates the composition of the supernatant fluid of fresh ascitic fluid which was centrifuged immediately

TABLE I.

COMPOSITION OF SUPERNATANT FLUID

#	pH	Na	K	Cl	CO_2	P	Total cation	Total anion	Undeter. anion
1.	-	152.6	7.2	115.0	-	-	160.0	-	-
2.	6.89	152.0	4.0	118.0	15.5	-	159.0	139.2	19.8
3.	6.71	138.0	7.0	112.0	29.6	-	145.0	148.3	-3.3
4.	7.14	158.6	8.5	115.2	-	-	157.0	-	-
5.	6.94	146.0	6.8	112.6	16.3	6.1	153.0	135.0	18.0
6.	6.97	160.0	9.0	119.6	17.7	6.4	169.0	142.0	27.0
7.	7.14	159.2	5.8	118.2	23.2	-	165.0	148.1	16.9
8.	7.06	169.0	7.1	112.8	20.8	-	176.0	140.3	35.7
9.	-	151.8	7.1	104.0	15.7	6.9	158.0	125.0	33.0

after removal from the host animal.

The average values in millimoles per liter are:

total cation	160 (145 - 176)
sodium	153 (138 - 169)
potassium	6.9 (4.0 - 9.0)
total anion	140
chloride	114.2 (104 - 119.6)
phosphate	6.7 (6.4 - 6.9)
CO_2	19.8 (15.5 - 29.6)
undetermined anion	20

If one excludes the aberrant values for chloride in sample #9 and for sodium in sample #8, the cationic and anionic composition of the supernatant is within relatively narrow limits. The composition of the undetermined anion is not known, but it is possible that organic acids might account for some of it. Two determinations of lactate and pyruvate were done by the method of Barker and Summerson. The pyruvate ranged from 14 γ/cc. to 20 γ/cc., and the lactate from 1200 γ/cc. to 2400 γ/cc. (10-20 meq/l.). The low pH in sample #3 cannot be accounted for at this time.

INTRACELLULAR COMPOSITION

The intracellular composition is indicated in Table II. It should be noted that these cells contain significant amounts of chloride and sodium. The values

TABLE II

INTRACELLULAR COMPOSITION

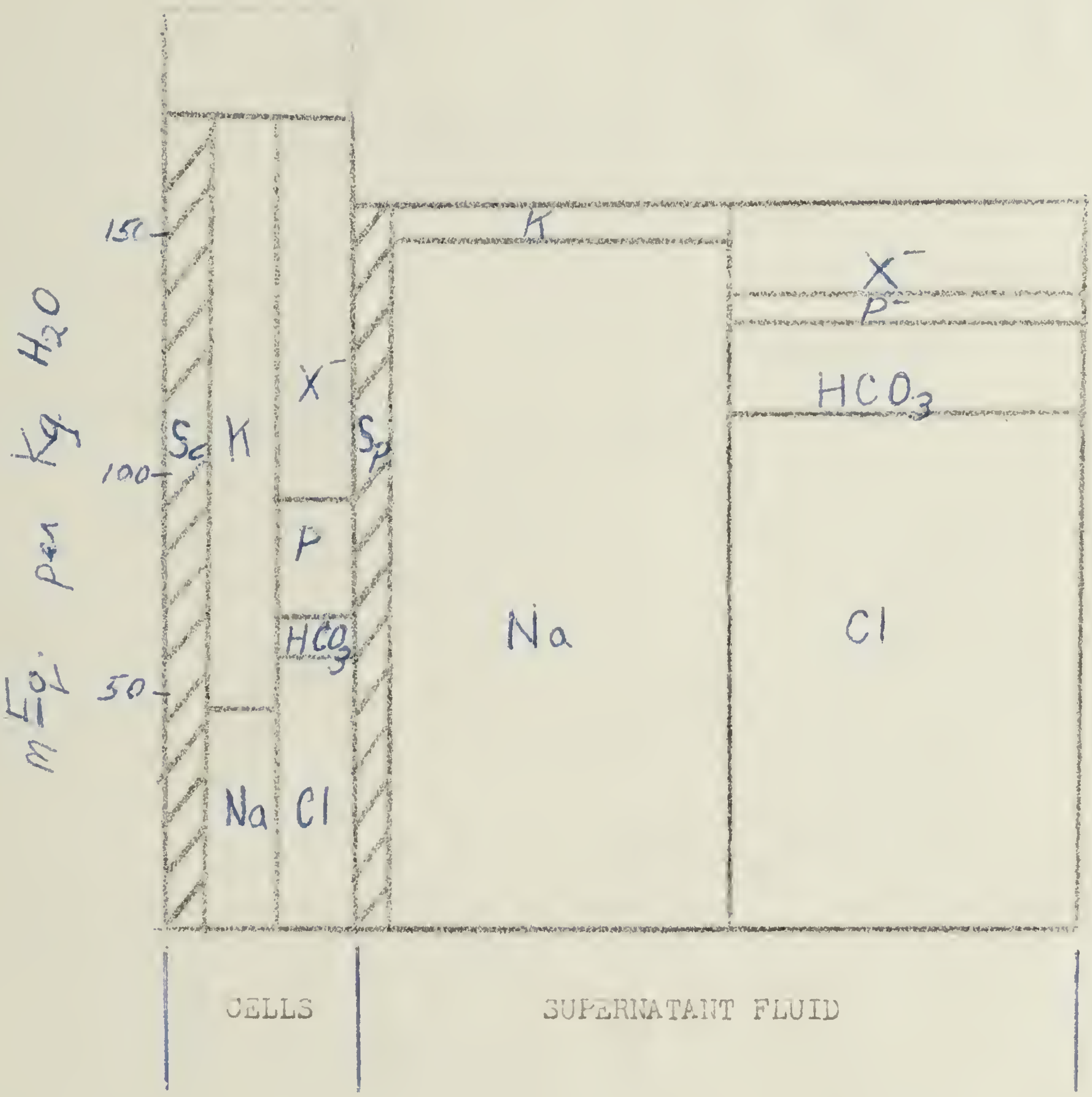
%HOH	Cl	Na	K	P	HCO_3*	H_2CO_3
79.7	59.0	47.0	131.0	23.1	8.1	9.3
80.3	57.0	48.4	129.0	6.6	11.9	13.1
77.0	58.5	49.0	126.0	27.2	12.4	13.6
84.1	46.7					

Values expressed per kilogram of cell HOH

* calculated when $p = 40mm \pm 5mm$

FIGURE III

ELECTROLYTES OF ASCITIC FLUID



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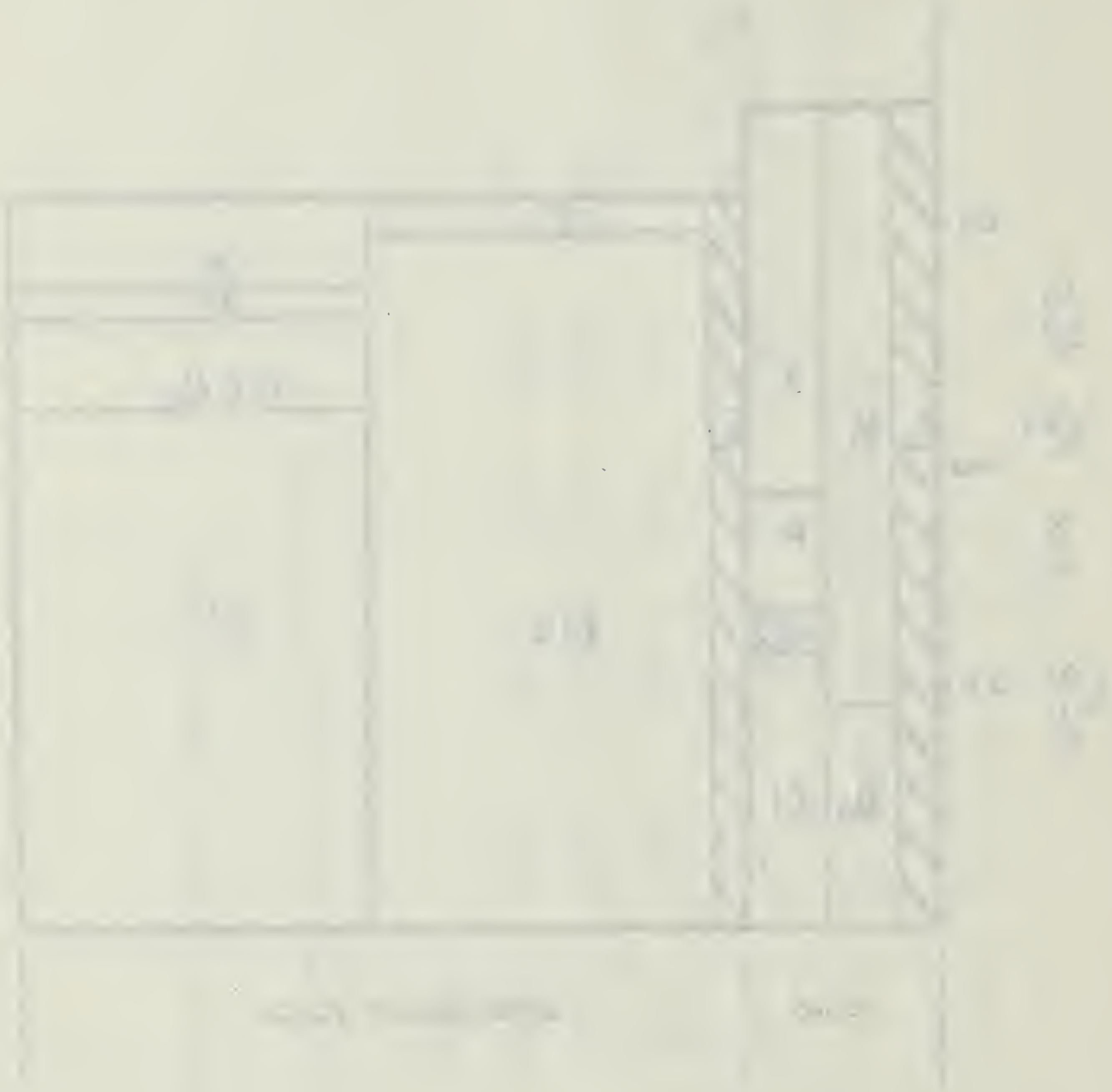

calculated for $[HCO_3]_c$ are based on the assumption that the ascitic fluid is in equilibrium with a p CO_2 of 40 ± 5 mm. The values obtained for $[HCO_3]_c$ compare roughly with the average value of 10 meq/l. calculated by Wallace and Hastings (29) for muscle cells. The valence of the intracellular phosphate ion cannot be determined, because the intracellular pH is not known. The composition of the ascitic fluid is shown in diagramatic form in Figure 2.

EFFECT OF HIGH TENSIONS OF CO_2

Calculations:

The amount of H_2CO_3 in mM/l. may be calculated by the formula (1):

$$\begin{aligned} \text{mM } H_2CO_3 &= \frac{P}{760 \times .02226} \\ &= .0591 \alpha P \\ &= .0591 \times .5290 \times P \\ &= .031P \end{aligned}$$

α refers to the solubility coefficient for CO_2, and P refers to the CO_2 concentration of a gas mixture in terms of pressure.

P is calculated by:

$$P\ CO_2 = (P_{air} - P_{water}) \times \frac{\%CO_2 \text{ in dry air}}{100}$$

where P_{air} equals the total gas pressure, (barometric pressure) and P_{water} equals the vapor tension of water at the temperature employed.

The factor .0591 is used to convert the final value to the millimolar concentration of H_2CO_3. The use of .5290 for the α of ascitic fluid will be discussed later.

The calculation of $[BHCO_3]_s$ from the CO_2 content and P can be determined by the formula:

$$\begin{aligned} mM\ BHCO_3 &= CO_2 - .0591\alpha P \\ &= CO_2 - .031\ P \end{aligned}$$

The concentration of intracellular bicarbonate, $[HCO_3]_c$, is made by the formula:

$$[HCO_3]_t = [HCO_3]_s \times V_s + [HCO_3]_c \times V_c$$

as discussed previously.

Results:

Despite high tensions of CO_2 no shift of chloride intracellularly was observed. The shift of chloride from the intracellular compartment observed in experiment #2 is difficult to explain.

Further, there were no consistent shifts of cations observed. The results calculated for $[HCO_3]_c$ will be discussed later.

Experiment #3 was carried out after the hematocrit of the ascitic fluid had been raised by removing supernatant fluid and resuspending the cells. It was hoped that decreasing the volume of supernatant fluid would accentuate

TABLE III

EFFECT OF HIGH TENSIONS OF CO_2

Hct	pH	$\%CO_2$	$\%O_2$	H_2CO_3	$[HCO_3]_{t\ w}$	$[HCO_3]_{s\ w}$	$[HCO_3]_{c\ w}$	Cl_s	Cl_c	Na_s	Na_c	K_s	K_c
EXPERIMENT #1.													
17.3	7.03	5.5	17.3	1.29	13.9	15.1	8.1	109.8	57.0	136.0	--	14.7	--
17.1	6.54	32.7	15.8	7.05	11.6	14.6	-2.9	110.0	56.0	136.0	--	14.7	--
EXPERIMENT #2.													
18.0	7.00	5.3	--	1.24	8.5	11.3	-3.9	96.8	63.3	132.0	--	11.2	--
18.1	6.32	47.0	--	11.04	8.5	10.6	-1.1	103.8	56.3	148.0	--	10.1	--
EXPERIMENT #3.													
42.1	--	5.6	18.1	1.32	13.8	15.2	11.9	109.0	--	141.0	--	14.6	--
43.0	--	40.0	18.9	9.44	9.7	12.9	5.4	110.0	--	142.0	--	14.4	--

any shift of ions. However, no shifts of electrolytes were observed.

Table IIIb indicates the "chloride shift" obtained for human erythrocytes by the methods outlined above. It should be noted that with an increase in CO_2 tension, there is an increase in hematocrit, a decrease in serum chloride, and no consistent shifts of cations.

TABLE IIIb

"CHLORIDE SHIFT" FOR ERYTHROCYTES

Hct	%CO_2	pH	Cl_s	Na_s	K_s	$[CO_2]_t$
43.6	6.5	7.39	104.2	151	5.0	
47.6	40.0	6.60	96.8	152	4.2	
44.7	3.3	—	105.6	149	4.12	
46.2	16.5	—	104.0	152	4.28	
43.4	13.0	7.28	105.6n	150	3.4	24.9
47.6	40.0	7.06	102.0	148	4.2	33.0

DETERMINATION OF α

CALCULATIONS

can be determined by the formula (1):

$$\alpha = \frac{760}{P} \times \frac{[CO_2]}{100} \times \frac{V_r}{V_{37^\circ}}$$

P equals the concentration of gas in terms of pressure, as discussed previously. $[CO_2]$ refers to the volume percent of CO_2 of the solution at room temperature. The ratio $\frac{V_r}{V_{37^\circ}}$ is a factor to correct for the change in volume of a solution as it cools from 37° to room temperature.

α^o refers to the solubility coefficient of CO_2 per gram of water of the solution. This value can be determined by the formula:

$$\alpha^o = \frac{760}{P} \times \frac{[CO_2]}{100(H_2O)}$$

(H_2O) represents the grams of water per cc. of solution at room temperature.

RESULTS

Using the experimental techniques described above, the α of distilled water was determined to be .5456, a figure which is within the experimental range determined by Van Slyke et. al. (27). The average value of six determinations of α of whole ascitic fluid was found to be .5290, a figure

TABLE IV

DETERMINATION OF α

#	BP	%CO_2	pCO_2	CO_2	α	α°
water	765.6	99.14	710.6	51.02	.5456	.5456
1.	751.2	99.11	696.9	48.55	.5292	.5571
2.	763.0	99.17	709.1	49.94	.5338	.5611
3.	762.0	99.12	707.7	48.86	.5247	.5523
4.	756.0	99.11	701.7	48.15	.5215	.5489
5.	754.2	99.15	700.2	49.86	.5411	.5695
6.	754.0	99.17	700.1	48.23	.5236	.5512

average α = .5290

average $\overset{\circ}{\alpha}$ = .5567

higher than the .510 value obtained for serum by Van Slyke, and 97% of the value for distilled water. According to Van Slyke (27), the deviations of the solubility of CO_2 in serum from the solubility of CO_2 in water are related to several factors. Salts depress the solubility about 3% by two mechanisms; they lower the solvent power of the water for CO_2 and to a lesser extent displace a small amount of water. Proteins depress the solubility of CO_2 several percent by displacing water. On the other hand, lipoids, because of their own high solvent power for CO_2, raise the solubility. In normal serum the combined effect of these factors is to reduce the solubility to 93-94% of the solubility of water. However, it has been observed that the solubility of CO_2 in lipemic serum often exceeds that in water. It would seem, then, that ascitic fluid must be either **very** low in salts and proteins or high in lipoids. It has been shown that the salt content of the ascitic fluid is at least equal to that of serum. However, no protein or lipoid determinations were done. It should be noted that the value obtained for ascitic fluid is the same as the value obtained for a solution containing only salts. Since some protein must be present in the ascitic fluid, the lipoid content of ascitic fluid must be higher than that of serum.

DETERMINATION OF pK'

CALCULATIONS

The pK' of an unknown fluid can be determined by the formula (1):

$$pK' = pH - \log \frac{[CO_2] - .0591\alpha P}{.0591\alpha P}$$

for ascitic fluid this formula can be applied as:

$$pK' = pH - \log \frac{[CO_2]_W - .031P}{.031P}$$

$[CO_2]_W$ refers to the CO_2 content of the water phase of the ascitic fluid.

RESULTS

Table V indicates the results of pK' calculations for the supernatant fluid. The average value is 6.07, slightly lower than the value 6.10 calculated for human serum by Hastings et. al. (30).

Table V_a lists the values computed for the pK' of the whole ascitic fluid. It should be pointed out that the reported values are probably in error because the intracellular pH cannot be measured.

It is also conceivable that the pCO_2 values within the cell are considerably higher than those determined by calculation from the CO_2 tension within the tonometer,

TABLE V.

DETERMINATION OF pK* OF SUPERNATANT FLUID

#	BP	$\%CO_2$	pCO_2	CO_{2s}	CO_{2w}	.03Ip	"-log()"	log	pH	pK
I.	756	32.7	246	2I.68	22.74	7.05	2.23	.3483	6.54	6.I9
2.	756	5.5	4I.6	I6.4	I7.3	I.29	I2.99	I.0932	7.03	5.94
3.	756	47.I	356.I	2I.6	22.64	II.04	I.498	.I755	6.32	6.I4
4.	756	5.34	40.I	I2.22	I2.86	I.24	9.452	.9755	7.00	6.02

$$* \; pK = pH - \log \left(\frac{[CO_2]_w - .059I\alpha_p}{.059I\alpha_p} \right)$$

$$.059I\alpha_p = .03Ip$$

TABLE Va

DETERMINATION OF pK OF WHOLE FLUID

#	BP	$\%CO_2$	pCO_2	CO_{2t}	CO_{2w}	.03Ip	"-log()"	log	pH	pK*
I.	765.5	5.90	45.I8	IO.97	--	I.40	6.836	.8348	6.68	5.84
2.	76I.5	5.65	43.02	I4.54	--	I.33	9.932	.9970	6.80	5.80
3.	762.I	5.28	40.25	II.60	--	I.25	8.280	.9I8	6.63	5.7I

*Calculation of pK as in Table V.

because the cells may be producing CO_2 as a product of their metabolism.

CALCULATION OF INTRACELLULAR pH

Wallace and Hastings (29) have used the Henderson-Hasselbach equation for calculating intracellular pH in the following manner:

$$pH_c = pK' + \log \frac{[HCO_3]_c}{[H_2CO_3]_c}$$

$$= pK' + \log \frac{[CO_2]_c - [H_2CO_3]_c}{[H_2CO_3]_c}$$

pK' of the intracellular phase has been investigated by Danielson et. al. (30). These authors found that the presence of tissue protein does not influence the value of pK' of carbonic acid when the values are computed as moles per kilogram of water. One would seem justified, then, to use the determined value of pK', 6.07, for ascitic fluid.

Wallace and Hastings (29), in their discussion of $[H_2CO_3]_c$, assume equality between the CO_2 content of the intracellular and extracellular phases. However, the solubility coefficient of CO_2 for the water of the intracellular phase used by these authors was .592 cc. per gram of intracellular water. This figure was arrived at by Danielson et. al. (30) empirically by correcting for the

amount of protein present in the intracellular fluid. No correction was made for the amount of lipoid that might also be present. With present experimental techniques, it is not possible to measure directly the intracellular α because the fluid must be acidified to a pH of 3.5 before equilibration, in order to drive off any bound CO_2. This low pH undoubtedly kills the cells.

It is possible that the intracellular α might be approached by progressively increasing the percentage of cells in the test fluid. By this method, it might be possible to extrapolate to a point where the test fluid is 100% cells, and arrive at a value for α. This type of experiment has not been adequately evaluated; so, for want of a better value, these calculations of pH_c were made using the value for α^o, .5567.

When the pCO_2 was about 40 mm., the average pH_c was 6.97. It should be noted that when the pCO_2 was raised, pH_c dropped quite low. This phenomenon will be discussed later.

FIGURE VI

INTRACELLULAR pH

$\%CO_2$	pCO_2	H_2CO_3	$[CO_2]_c$	log( )	log =	pH_c
5.5	41	1.35	9.4	5.63	.751	6.82
5.6	43	1.41	13.2	8.43	.926	7.00
5.4	40	1.32	13.1	9.08	.958	7.03
5.4	40	1.32	13.6	9.46	.976	7.05
					Average value	6.97
40.0	304	10.03	14.8	.48	9.68-10	5.75

$$pH_c = pK' + \log \frac{[HCO_3]_c}{[H_2CO_3]_c}$$

DISCUSSION

Much of the work presented in this paper was frankly exploratory in an effort to determine if the Ehrlich mouse ascites tumor was a favorable medium for the investigation of problems of electrolyte equilibrium. The ease with which these cells may be obtained, the in vitro stability of these cells, and the relatively uniform electrolyte composition, all suggest that these cells might be useful for investigation of various osmotic and metabolic phenomena in actively metabolising, nucleated cells. The values obtained for α and pK' are additional data which help to delineate the basic characteristics of these cells.

With these preliminary studies completed, an attempt was made to incorporate these cells into an experimental situation. The evaluation of the effects of high tensions of CO_2 was chosen for several reasons. This experiment was well-documented for blood, and the results should be relatively clear-cut. Further, the cells would not be subject to too many unphysiological stimuli. Finally, the possibility of chloride exchanges between tissue cells and their surrounding fluids have never been adequately evaluated.

According to the theory depicted in Figure 1, if

the concentration of bicarbonate rose in one compartment, either intracellularly or extracellularly, an exchange for chloride of the other compartment might take place. Pitts and his co-workers (10) suggested in 1952 that when the extracellular bicarbonate of a nephrectomized dog is raised by the infusion of $NaHCO_3$, some of the added extracellular bicarbonate exchanges for cellular chloride. On recalculating their data, using radiosulfate to measure the extracellular space rather than inulin, these authors (6) found that very little, if any, chloride-bicarbonate exchange occurs. When respiratory acidosis occurs or is induced, the in vivo conditions are not unlike those which exist during the "chloride shift" experiments. Here again the status of the tissue contribution to buffering within the extracellular fluid is not clear. Pitts and his co-workers (7) feel that several adjustments occur. Sodium and potassium shift to the extracellular fluid, chloride shifts to the erythrocytes, and alterations of the organic acid of the extracellular fluid occur. These authors also suggest that some chloride may shift across some other extracellular boundry besides the erythrocyte.

In this series of experiments, no significant alterations in the pattern of extracellular and intracellular composition occurred, despite the use of high tensions of CO_2. Further, if any intracellular buffering does occur, it must be to a limited extent, because there are significant

drops in the pH of the tumor fluid.

In an isolated buffer system, the addition of CO_2 results in an increase of carbonic acid, and an increase of bicarbonate. In the intact cell, this may not occur. Wallace and Hastings (20), while studying muscle cells, observed that the intracellular bicarbonate did not rise, despite physiologic elevations of the $BHCO_3$ content of the serum. In this cell system, with high tensions of CO_2, the intracellular bicarbonate actually decreased. It is almost as if alterations in active cellular metabolism interfere with the buffering ability of carbonic acid systems.

It is possible that under the gas tensions employed in these experiments anaerobic glycolysis occured with the production of large amounts of organic acid. The large amounts of organic acid could depress the level of bicarbonate to the values found in these experiments. If this phenomenon occurs, the intracellular pH must sink to extremely low values (to about 5.7 in experiment #3). If this supposition is correct, under the conditions imposed in these experiments, the intracellular buffers fail completely in their maintenance of hydrogen ion concentrations, and allow the intracellular pH to fall even more than extracellular pH.

Any further attempt at interpretation must await more experimentation.

SUMMARY

1. The cells of the Ehrlich ascites tumor of mice are a suitable medium for the investigation of problems of electrolyte equilibrium.

2. The extracellular and intracellular composition, the solubility coefficient of CO_2, the pK', and the intracellular pH are all defined within relatively narrow limits.

3. When the ascitic fluid is exposed to high tensions of CO_2, no consistent exchanges of ions are observed.

4. With high tensions of CO_2, the intracellular bicarbonate was observed to decrease. This phenomenon suggests that large amounts of organic acid are being formed, which depress intracellular bicarbonate and, consequently, intracellular pH.

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